# Analysis of Genetic Diversity of *Mobula* sp. in Cilacap Using RAPD (Random Amplified Polymorphic DNA) Markers

.


## ABSTRACT

Devil rays (*Mobula* sp.) are in an endangered status, so conservation measures are needed. One of the first steps is to identify the level of genetic diversity.This study aims to determine the genetic diversity of mobula in Cilacap based on DNA bands of RAPD (Random Amplified Polymorphic DNA) polymorphism genetic markers. This research was conducted in September 2021 – June 2022. Sampling was carried out at PPS Cilacap and molecular analysis was carried out at the Biotechnology Laboratory building 3, Faculty of Fisheries and Marine Sciences, Padjadjaran University. The research procedure began with preservation of the devil rays (*Mobula* sp.) sample, isolation of sample DNA, electrophoresis of isolated DNA results, calculating DNA purity, DNA amplification, electrophoresis of DNA amplification results, and data processing and analysis. DNA isolation was carried out using the Promega Kit method. The electrophoretic results of DNA isolation from the three mobula rays samples showed quite good results. Band samples CIL 10 and CIL 9 looked thick and did not smear, while C13 produced bands and there was a visible smear. The results of calculating the purity of DNA isolated from mobula rays CIL 9, CIL 10, CIL 13 have a purity value of 1.94, 1.96, 1.80. Results The highest concentration of DNA was CIL-13 sample with a value of 452.90 ng/μL while the lowest concentration was CIL-10 which was 419.90 OPE-01 resulting in 10 out of 20 visualized polymorphic bands. The similarity index of CIL-9 to CIL-10 in the OPE-01 primer was 40% while the similarity index of CIL-9 and CIL 10 to CIL-13 was 35%. The average similarity index shows that the genetic diversity of the Mobula species is moderate. Morphologically, CIL-09 is a species of Mobula thrustoni, CIL 10 is a species of Mobula mobular, and CIL-13 is a species of Mobula tarapacana. CIL-9 (*M. Thrustoni*) has a closer similarity index to CIL-10 (*M. Mobular*) than CIL-13 (*M. Tarapacana*). OPE-01 primer is good for analyzing diversity in mobula rays (*Mobula* sp.). OPE-01 has 10 out of 20 visualized polymorphic bands.



*Keywords: Amplification, M. mobular, M. thrustoni, M. tarapacana*


## 1. INTRODUCTION

Cilacap Regency is a district in Central Java Province in the southern part of Java Island. Cilacap Regency has a tropical climate where the rainy season is longer and the dry season is throughout the year. The highest rainfall occurred in September with 661.9 mm with 29 rainy days. The lowest rainfall occurred in February of 67.5 mm [1]. Fishing efforts in coastal waters are carried out to a distance of ± 12 nautical miles from the shoreline to a depth of 3-100 m or at the boundaries of the territorial waters of Indonesia.

The Mobula genus is currently facing the problem of a high rate of extinction due to overfishing triggered by market demand for mobula meat and skin [2]. Several places in Indonesia have caught stingrays, for example in Central Java in 2019 it reached 5,763.16 tons and for the mobula genus at Cilacap Fish Auction Ocean Fishing Port in 2019 it reached 5.83 tons [3]. According to [4], the stingrays that landed at Cilacap Fish Auction

Ocean Fishing Port were *Mobula japanica*, *M. tarapacana*, *M. thursoni*, *M. cf khulii*, and *Manta birostris* as by-catch from drift gill nets.

Mobula ray from the genus Mobula are animals that come from the Mobulidae family. Based on [5], states that species in the genus Mobula in Cilacap waters are included in the endangered category such as *M. thurstoni*, *M. Mobular* and *M. Tarapacana* [6, 7, 8, 9, 10, 11]. Small populations are more susceptible to several adverse genetic effects so these animals experience a decrease in their ability to evolve or adapt to changing environments [12].

Mobula rays are found in productive coastal waters with regular up-welling processes, groups of small islands, around seamounts, and offshore waters. [13]. The condition of the aquatic environment determines the location of the movement of stingrays. The relationship between populations in one location with another can be known through genetic markers [14].

A genetic marker is a piece of genetic material (usually DNA) that can be easily identified to differentiate between cells, individuals, populations, or species [15]. Nucleotide base sequences that vary between species can be used as specific markers that provide knowledge about phylogenetic relationships to overcome doubts in systematics. Genetic marker Random Amplified Polymorphic DNA (RAPD) is a DNA genome amplification based on the PCR technique [16]. Attachment segments with different DNA fragment lengths are assumed to follow Mendelian inheritance [17]. The RAPD marker has advantages including not requiring nucleotide base sequence information to read [18,19], the primers used are commercially available, do not require information about target DNA sequences or gene organization [20], and is fast to do [21]. The disadvantage of RAPD is that it is not able to distinguish between homozygous and heterozygous loci, so it requires analysis of the nucleotide base sequence using high-resolution techniques [22]. RAPD analysis can be used to see genetic diversity based on the level of polymorphism, inheritance patterns, check the origin of populations, and help define a species [23]. These markers are widely used in identifying genetic diversity at the interspecies and interspecies levels [21].

The diversity of genetic resources of a population has factors that influence the response of a population to natural and artificial selection. Populations with high genetic diversity have a better chance of survival [24]. This is because each gene has a different response to environmental conditions so if an individual has various kinds of genes in a population, there is a chance of response to environmental changes [25].

The existence of stingrays is in endangered status, therefore it is necessary to carry out conservation measures. One of the first steps in supporting conservation efforts is the need to identify the level of genetic diversity. Research on the genetic diversity of stingrays from the genus Mobula in Cilacap using the RAPD technique has not been carried out. Therefore, it is necessary to research the analysis of the genetic diversity of the genus Mobula stingrays in Cilacap waters using the RAPD technique. This study aimed to determine the genetic diversity of stingrays in Cilacap based on DNA bands of RAPD (Random Amplified Polymorphic DNA) polymorphism genetic markers.

## 2. METHODOLOGY

### 2.1 Time and Place of Research

This research was conducted in September 2021 – June 2022. Sampling was carried out at PPS Cilacap. Devil rays that used for sampels are by catch product. Molecular analysis was

carried out at the Biotechnology Laboratory building 3, Faculty of Fisheries and Marine Sciences, Padjadjaran University..

The methods are included in the survey method with quantitative and qualitative descriptive analysis. Quantitative data were obtained from DNA purity calculations while qualitative data were obtained from the appearance of visualized bands from DNA amplification electrophoresis results.

## 2.2 Procedures

The research procedure began with preservation of the devil rays (*Mobula* sp.) sample, isolation of sample DNA, electrophoresis of isolated DNA results, calculating DNA purity, DNA amplification, electrophoresis of DNA amplification results, and data processing and analysis.

### 2.2.1 DNA Isolation

DNA isolation was carried out in two ways, Wizzard Genomic Purification Kit (Promega) [26].

The purity of isolated DNA was measured with a Multimode Reader Infinite 200 PRO NanoQuant spectrophotometer. The purity of DNA is obtained from the division between A260 and A280. The isolated DNA sample was diluted 50× Nuclease Free Water into a blank. The absorbance was measured at a wavelength (λ) of 260 nm and 280 nm and then the absorbance was recorded for each wave. The results for purity and quantification were recorded.

### 2.2.2 DNA Amplification

DNA amplification was carried out by [27] method. The primer used was OPE-01 (CCCAAGGTCC) [27].

12.5 µl of MyTaqTM HS Red Mix (Bioline), 9.5 µl of NFW, 1 µl of primer, and 2 µl of DNA sample, put into a 0.2 ml microtube then the solution was centrifuged for 30 seconds at 1,000-speed rpm. Amplification consists of several stages, namely pre-denaturation (94°C, 60s, 3 cycles), pre-annealing (35°C, 60s, 3 cycles), pre-extension (72°C, 120s, 3 cycles), Denaturation (94°C, 10s, 37 cycles), annealing (40°C, 30s, 37 cycles), extension (72°C, 120s, 37 cycles), post extension (72°C, 300s, 1 cycle) and cooling (4°C, 60s, 1 cycle) [27].

### 2.2.3 Electrophoresis of DNA Isolation and Amplification

Electrophoresis is a separation method that utilizes the electric field generated from the electrodes to separate DNA molecules which will later produce DNA bands that can later be analyzed based on the presence or absence of bands in the sample.

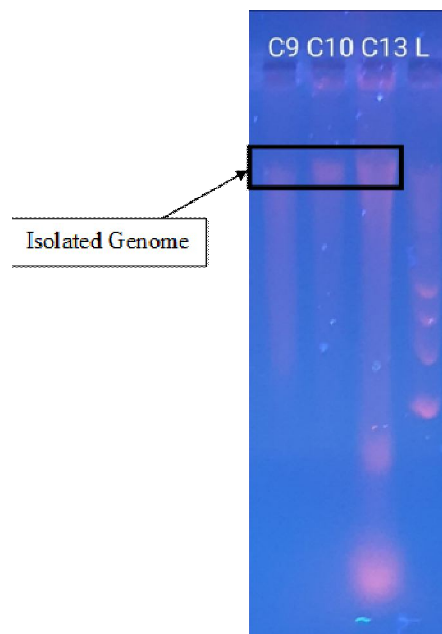
Agarose powder was weighed as much as 0.4 grams and dissolved with 40 ml of TAE 1X. The solution was heated in the microwave for three minutes. When finished, the agarose solution was allowed to stand until warm and 0.6 µl of red gel was added and homogenized. The agarose gel mold was prepared, then the agarose solution was poured into the mold. The agarose gel is left for 15-30 minutes to freeze. The agarose gel was put into the electrophoresis tank which already contained the TAE running buffer solution until it was completely submerged. Each sample was entered successively based on the primer into the agarose gel wells until all samples occupied the wells. The composition of the isolated sample was 4 µl of DNA isolation sample, and 2 µl of loading dye. The composition of the amplified sample is 3 µl. The composition of the marker DNA ladder of 100 bp was 2 µl. The Mupid-Exu electrophoresis machine was set at to voltage of 70 v, for 30 minutes for DNA isolation results, and a voltage of 70 v, for 90 minutes for DNA amplification results. Agarose

121 gel was visualized by placing it on a UV transilluminator. The visualization results are
122 documented for further analysis.

123 **2.2.4 Data Analysis**
124 DNA amplification produces random DNA fragments with RAPD primers. The length of the
125 separated fragments using RAPD primers can be determined from the migration distance of
126 the DNA bands from the wells and compared with the markers used using the CorelDraw
127 application.

128 Migration distance data is then entered into the excel application and analyzed to obtain
129 band sizes. The bands visualized on the agarose gel are translated into a binary matrix.
130 Bands that appear are defined as one (1) and bands that do not appear are defined as zero
131 (0). Binary data is entered into the excel application with excel format 1997-2003. Binary
132 matrix data that has been processed in the excel application is then processed in the Ntedit
133 application. Data that has been processed in the Ntedit application is entered into the NTSys
134 application to obtain a dendrogram tree. The genetic similarity index is calculated based on
135 the Dice Coefficient formula [28].

136 ## 3. RESULTS AND DISCUSSION
137
138 ### 3.1 DNA Concentration and Purity
139
140 The electrophoretic results of DNA isolation from the three stingray samples showed quite
141 good results but there were still smears. The best band results are samples CIL 10 with code
142 C10 and CIL 9 with code C9. Band samples CIL 10 and CIL 9 looked thick and did not
143 smear, while C13 produced bands and there was a visible smear. CIL 13 carried out a
144 different isolation method, namely the CTAB isolation method. The CTAB isolation method
145 was performed because the band on the Promega Kit isolation method did not appear. This
146 is because the storage time of the sample has reached 1 year. According to [29], storage of
147 samples using glycerol alcohol will decrease by around 60-80%.




148 .

149 **Figure 1. DNA electrophoresis results**
150 *L=Ladder 1kb, C9=CIL-9, C10=CIL-10, C13=CIL-13*
151
152 The results of calculating the purity of DNA isolated from stingrays CIL 9, CIL 10, CIL 13
153 have purity values of 1.943, 1.958, 1.803. These DNA purity value shows high results and
154 can be used for PCR. Purity results are good if they range from 1.8 to 2.0 [30,31]. The purity
155 value of DNA isolation above 2 indicates that it still contains RNA, while a purity result of less
156 than 1.8 indicates that the DNA extract still contains protein [32]. UV light with a wavelength
157 of 260 nm can be absorbed by ds-DNA, while light with a wavelength of 280 nm can be
158 absorbed by protein or phenol contaminants [33].

159 Results The highest concentration of DNA was in the CIL-13 sample with a value of
160 452.8988 ng/µL. This was seen in the electrophoresis results (Figure 1) where the band was
161 the thickest among others. The lowest concentration was CIL-10 which was 419.8988 ng/µL.
162 This difference in concentration values can be caused by differences in isolation methods.
163 The CTAB isolation method has the advantage of being able to perform strong lysis and
164 facilitate protein precipitation so it can produce high concentrations of DNA [34].

165

166 **Table 1. Results of Quantification of DNA Concentration and Purity**

| No | Sample | A260 | A280 | Consentration (ng/µL) | Purity of DNA |
|---|---|---|---|---|---|
| 1 | CIL-9 | 0,17256 | 0,088796 | 431,3988 | 1.943 |
| 2 | CIL-10 | 0,16796 | 0,085796 | 419,8988 | 1.958 |
| 3 | CIL-13 | 0.,18116 | 0,100496 | 452,8988 | 1.803 |

167
168 Isolation using the Wizard Genomic DNA Purification kit Brand Promega and CTAB from
169 samples of Mobula rays that had been preserved with glycerol alcohol could be tested to
170 produce good DNA. DNA samples can be tested for the next process, namely DNA
171 amplification because testing the results of isolation qualitatively and quantitatively shows
172 pure DNA results.

173 ## 3.2 Genetic Diversity of Mobula

174 Fragments appear in all primers used for amplification of 3 samples. The primer used in this
175 DNA amplification is OPE-01. OPE-01 produces 20 bands. All bands amplified using prunes
176 were analyzed further.

177 The size of the DNA fragments amplified in the OPC-20 primer was, OPE-01 was 390 - 5078
178 bp. According to [35], the base length (bp) found in RAPD DNA fragments in fish ranges
179 from 200 - 1500 bp, whereas according to [26], the base length in fish ranges from 300-1500
180 bp.

181 **Table 2. OPE 01 primer polymorphic and monomorphic bands** (-- = Polymorphic band, --
182 *=monomorphic bands)

| Fragment Range (mm) | CIL-9 | CIL-10 | CIL-13 | Band Size (bp) |
|---|---|---|---|---|

| Fragment Range (mm) | CIL-9 | CIL-10 | CIL-13 | Band Size (bp) |
|---|---|---|---|---|
| 203 | | --* | | 5078 |
| 239 | | | --* | 2681 |
| 252 | -- | -- | | 2226 |
| 274 | | | --* | 1692 |
| 287 | -- | -- | | 1467 |
| 303 | | | --* | 1252 |
| 317 | | --* | | 1104 |
| 333 | | -- | -- | 970 |
| 348 | | | --* | 867 |
| 362 | | -- | -- | 788 |
| 373 | | -- | -- | 735 |
| 386 | | -- | -- | 680 |
| 391 | --* | | | 661 |
| 407 | -- | | -- | 606 |
| 412 | -- | -- | | 591 |
| 433 | -- | -- | -- | 534 |
| 448 | -- | | -- | 499 |
| 463 | | --* | | 469 |
| 477 | --* | | | 444 |
| 514 | --* | | | 390 |

183

184 OPE-01 produced 10 out of 20 visualized polymorphic bands. Polymorphic bands are bands
185 that are not present in all samples [36], while monomorphic bands are bands that exist in
186 other samples so that they do not have variations [37]. According to [36], differences in
187 banding patterns can describe genetic differences in samples so that unique and different
188 banding patterns can indicate genetic variation. The size and number of amplified fragments
189 depend on the nucleotide sequence of the DNA source [38]. The selection of primers in the
190 RAPD method can increase polymorphism [26].

A dendrogram tree is a tree that describes a kinship relationship between species [26]. According to [39], genetic diversity is closely related to the genetic distance where the greater the genetic distance the greater the individual genetic diversity in the population. Conversely, the smaller the genetic distance, the lower the genetic diversity. There was a dendrogram produced in this study based on the OPE-01 primer. Dendogram tree analysis uses the dice similarity index [28]. The dice similarity index is used to see similarities and compare one sample to another so that the higher the percentage, the more similar the two populations are. From the genetic similarity index can be analyzed the genetic diversity of the individual. There is a dendrogram produced in this study based on the OPE-01 primer.

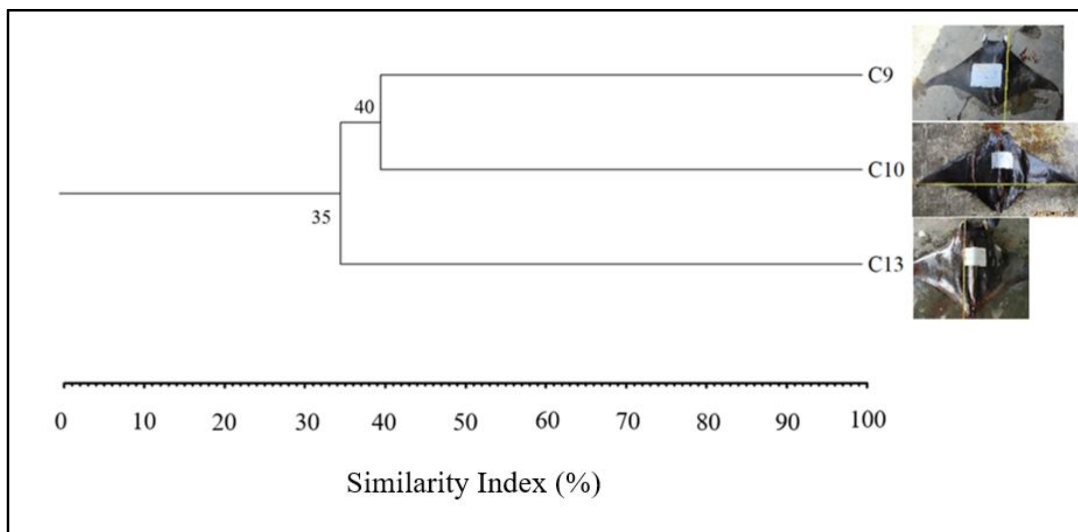



**Figure 2. Dendogram of stingray (Mobula sp.) based on OPE 01 RAPD marker**

The similarity index of CIL-9 to CIL-10 in the OPE-01 primer is 40%. This genetic distance is far away, therefore the genetic diversity is getting bigger. According to [38], the greater the genetic distance, the greater the individual genetic diversity in the population, conversely, the smaller the genetic distance, the lower the genetic diversity. At CIL 13 both primers have a similarity index of 35%.

According to [40], the value of genetic diversity ranges from 0.1 – 0.4 which is included in the low category, while values of 0.5 – 0.7 are classified as in the medium category, and 0.8 – 1.0 is in the high category. The average of the genetic diversity of this mobula ray is 62.5%, therefore the genetic diversity is moderate.

Morphologically, CIL-09 is a species of Mobula *thrustoni*, CIL-10 is a species of Mobula *mobular*, and CIL-13 is a species of Mobula *tarapacana*. According to [41], the morphology of the three species is described in table 21.

**Tabel 3. *Mobula* sp. Morphology**

| Species | Morphology |
|---|---|
| *Mobula mobular* | 1. wide body plate and slightly curved sharply<br>2. the tip of the dorsal fin is white |

| Species | Morphology |
|---|---|
| | 3. there is a thorn at the base of the tail<br>4. spiracles shaped like a small slit and rounded elongated, above the end of the body plate |
| *Mobula thrustoni* | 1. short head shape, less than 16% of body width<br>2. the tip of the dorsal fin is white<br>3. flat tail base<br>4. Slightly rounded spiracles, located under the ends of the body plates |
| *Mobula tarapacana* | 1. the body plate is not too wide and sharply curved<br>2. The dorsal fin is plain, the tip is not white<br>3. no spines at the base of the tail<br>4. spiracles like slits and elongated round, located above the end of the body plate |

The principle of RAPD is to randomly attach a single primer to DNA [42]. Most of the sample DNA is present in the nucleus, but a very small amount of DNA is also present in the mitochondria [43]. This grouping is similar to the study [44], where Mobula *mobular* is closer to Mobula *tarapacana* than Mobula *thrustoni* in mitochondria however, in the nucleus Mobula *mobular* is closer to Mobula *thrustoni* than Mobula *tarapacana*.

## 4. CONCLUSION

Based on the results of the discussion of the research that has been done, the conclusion obtained is that the average similarity index 62.5% indicates that the genetic diversity of the Mobula species is moderate. Morphologically, CIL-09 is a species of Mobula thrustoni, CIL 10 is a species of Mobula mobular, and CIL-13 is a species of Mobula tarapacana. CIL-9 (*M. Thrustoni*) has a closer similarity index to CIL-10 (*M. Mobular*) than CIL-13 (*M. Tarapacana*). OPE-01 primer is good for analyzing diversity in devil rays (*Mobula sp.*). OPE-01 has 10 out of 20 visualized polymorphic bands.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239 **REFERENCES**
240


241 [1] Diskominfo Cilacap. Profil Kondisi Geografis Kabupaten Cilacap. Pemerintah Kabupaten
242 Cilacap. 2017. Accessed 29 October 2022 Available:https://cilacapkab.go.id/v3/kondisi-
243 umum/. Indonesian

244 [2] Putranto Y. Stategi Pengawasan, Identifikasi Kapal Dan Peran Kelompok Masyarakat
245 Nelayan Dalam Pengawasan Kapal Penangkap Ikan Pari Di Perairan Cilacap. Dinamika
246 Journal : Pengabdian Masyarakat. Indonesian. 2019;1(1): 31–39.

247 [3] Kementerian Kelautan dan Perikanan. Produksi Perikanan Provinsi 2019. 2019.
248 Accessed 29 October 2022
249 Available:https://statistik.kkp.go.id/home.php?m=prod_ikan_prov&i=2#panel-footer.
250 Indonesian

251 [4] Dharmadi, Sunarno MTD, Edrus IN. Perikanan Dan Aspek Biologi Ikan Pari Lampengan,
252 Mobula japanica Di Perairan Selatan Jawa. Bawal. Indonesia. 2011;3(6): 369–376.

253 [5] IUCN Red List. 2022. redlist species data Mobula. International Union for Conservation of
254 Nature and Natural Resources.

255 [6] Marshall A, Barreto R, Bigman J, Carlson J, Fernando D, Fordham S, et.al.. Mobula
256 thurstoni, Bentfin Devilray. The IUCN Red List of Threatened Species, e.T60200A1, 20.
257 2019

258 [7] Marshall A, Barreto R, Bigman J, Carlson J, Fernando D, Fordham, et.al.. Mobula
259 tarapacana, Sicklefin Devilray. The IUCN Red List of Threatened Species, e.T60199A1.
260 2019

261 [8] Marshall A, Barreto R, Carlson J, Fernando D, Fordham S, Francis M, et.al.. Mobula
262 mobular, Spinetail Devil Ray Amendment version. 8235. 2020

263 [9] Marshall A, Barreto R, Carlson J, Fernando D, Fordham S, Francis, M., et.al. Mobula
264 hypostoma. The IUCN Red List of Threatened Species, 8235(e.T126710128A896599.).
265 2019

266 [10] Marshall A, Barreto R, Carlson J, Fernando D, Fordham S, Francis M, et.al. Mobula
267 munkiana. The IUCN Red List of Threatened Species 2019, 8235, e.T60198A124450956.
268 2019.

269 [11] Marshall A, Barreto R, Carlson J, Fernando D, Fordham S, Francis, et.al. Mobula
270 eregoodoo (Longhorned Pygmy Devil Ray). 8235. 2020.

271 [12] Hasibuan FEB, Mantiri FR, Rumende RR. Kajian Variasi Sekunes Intraspesies dan
272 Filogenetik Monyet Hitam Sulawesi (*Macaca nigra*) Dengan Menggunakan Gen COI. Jurnal
273 Ilmiah Sains. Indonesian. 2017;17(1): 59.

274 [13] Hermansyah R. Kabar Terbaru Dari Ikan Pari Mobula. Direktorat Jenderal Pengawasan
275 Sumber Daya Kelautan dan Perikanan. 2018. Accessed 29 October 2022
276 Available:https://kkp.go.id/djpsdkp/ditppsdk/artikel/2785-kabar-terbaru-dari-ikan-pari-mobula.
277 Indonesian

278 [14] Aini NK, Mashar A, Madduppa HH, Wardiatno Y. Keragaman genetik mimi
279 (*Carcinoscorpius rotundicauda dan Tachypleus gigas*) di perairan Demak, Madura dan
280 Balikpapan berdasarkan penanda Random Amplified Polymorphic DNA. Jurnal Pengelolaan
281 Sumberdaya Alam Dan Lingkungan (Journal of Natural Resources and Environmental
282 Management). Indonesian. 2020;10(1): 124–137.

283 [15] Wirdateti, Wijayanti WS, Kuswandi CP. Penanda Genetik Tarsius (Tarsius spp.) dengan
284 Menggunakan Gen Cytochrome Oxidase I (COI) DNA Mitokondria (mtDNA) Melalui Metode
285 Sekuensing (Genetic Marker on Tarsier (Tarsius spp.) using Cytochrome Oxidase I Gene
286 (COI) of Mitochondrial DNA (mtDNA) through. Jurnal Biologi Indonesia. Indonesian.
287 2015;11(2): 275-284.

288 [16] Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms
289 amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research, 1990;
290 18(22): 6531–6535.

291 [17] Chauhan T, Rajiv K. Molecular markers and their applications in fisheries and
292 aquaculture. Advances in Bioscience and Biotechnology, 2010;01(04): 281–291.

293 [18] Iyengar A, Piyapattanakorn S, Stone DM, Heipel DA, Howell BR, Baynes SM, et.al.
294 Identification of microsatellite repeats in turbot (Scophthalmus maximus) and dover sole
295 (Solea solea) using a RAPD-based technique: Characterization of microsatellite markers in
296 dover sole. Marine Biotechnology, 2000;2(1): 49–56.

297 [19] Nebauer SG, Castillo-Agudo DL, Segura J. An assessment of genetic relationships
298 within the genus Digitalis based on PCR-generated RAPD markers. Theoretical and Applied
299 Genetics, 2000;100(8): 1209–1216.

300 [20] Liu Z. Aquaculture Genome Technologies. 1st ed. Victoria. Blackwell Publishing; 2007.

301 [21] Irmawati, Parawansa BS, Malina AC, Tassakka A, Baharuddin M, Larekeng SH.
302 Keragaman Genetik Ikan Kakap Putih Tipe Liar dan Domestikasi Menggunakan Metode
303 RAPD. Journal Fisheries and Marine Research. Indonesian. 2021;5(1): 99–105.

304 [22] Martono B, Udarno L. Keragaman Genetik Beberapa Genotipe Teh Berdasarkan
305 Penanda RAPD (Random Amplified Polymorphic DNA). Jurnal Tanaman Industri Dan
306 Penyegar. Indonesian. 2014;1(1): 1-6.

307 [23] Anggereini E. Random Amplified Polymorphic DNA (RAPD), Suatu Metode Analisis
308 DNA Dalam Menjelaskan Berbagai Fenomena Biologi. Biospecies. Indonesian. 2008;1(2):
309 73–76.

310 [24] Yusron E. Pemanfaatan keragaman genetik dalam pengelolaan sumberdaya hayati laut.
311 Oseana. Indonesian. 2005;30(2): 29–34.

312 [25] Kantun W, dan Mallawa A. Biologi Tuna Madidihang (*Thunnus Albacares*) 1st ed.
313 Gadjah Mada University Press. Indonesian; 2018.

314 [26] Hafidah R, Yustiati A, Mulyani Y, Suryadi IBB. Determination of Genetic Diversity of
315 Four Guppy Strains Using the Random Amplified Polymorphic DNA-PCR. Asian Journal of
316 Biochemistry, Genetics and Molecular Biology. 2021;8(1): 1–8.

317 [27] Faria VV, Rolim LS, Vaz LA, Furtado-Neto MA. Reevaluation of RAPD markers involved
318 in a case of stingray misidentification (Dasyatidae: Dasyatis). Genetics and Molecular
319 Research : GMR. 2012;11(4): 3835–3845.

320 [28] Budi IM, Mawardi A. Identifikasi Molekular Kekerabatan Genetik Kopi Wamena Berbasis
321 Marka Random Amplified of Polymorphic DNA (RAPD). Jurnal Biologi Papua. Indonesian.
322 2021;13(1): 8–18.

323 [29] Novita H, Mufidah T, Koesharyani I. Perbandingan Penggunaan Berbagai Preservasi
324 Rna Jaringan Dengan Rna Later , Alkohol , Dan Alkohol-. J. Ris. Akuakultur. Indonesian.
325 2009;4(3): 377–383.

326 [30] Bijay RS, Giri RT, Ram PC. DNA isolation and optimization of PCR protocol for ISSR
327 analysis of Girardinia diversifolia: A medicinal and economic plant species from Nepal
328 Himalaya. African Journal of Biotechnology. 2020;19(10):747-753

329 [31] Wu Q, Chen M, Buchwald M, Phillips RA. A simple, rapid method for isolation of high
330 quality genomic DNA from animal tissues. Nucleic Acids Research, 1995;23(24): 5087–
331 5088.

332 [32] Ghatak S, Muthukumaran RB, Nachimuthu SK. A simple method of genomic DNA
333 extraction from human samples for PCR-RFLP analysis. Journal of Biomolecular
334 Techniques, 2013;24(4): 224–231.

335 [33] Widyaputri T, Pratama AC, Cidiastuti D, Oktanella Y. Exploration of Polymorphism
336 Analysis of CD18 Gene with PCR- RFLP Technique in Friesian Holstein Crossbreed Dairy.
337 Advances in Animal and Veterinary Sciences, 2022;10(4): 935–941.

338 [34] Fitriya RT, Ibrahim M, Lisdiana L. Keefektifan Metode Isolasi DNA Kit dan CTAB/NaCl
339 yang Dimodifikasi pada Staphylococcus aureus dan Shigella dysentriae. LenteraBio.
340 Indonesian. 2015;4(1): 87–92.

341 [35] Liu ZJ, Li P, Argue BJ, Dunham RA. Random amplified polymorphic DNA markers:
342 Usefulness for gene mapping and analysis of genetic variation of catfish. Aquaculture,
343 1999;174(1–2): 59–68.

344 [36] Sinaga A, Putri LAP, Bangun MK. Analisis Pola Pita Andaliman (Zanthoxylum
345 acanthopodium D.C) Berdasarkan Primer OPD 03, OPD 20, OPC 07, OPM 20, OPN 09.
346 Jurnal Agroekoteknologi FP USU. Indonesian. 2017;5(1): 55–64.

347 [37] Muharam EG, Buwono ID, Mulyani Y. Analisis Kekerabatan Ikan Mas Koi
348 (Cyprinuscarpio koi) dan Ikan Mas Majalaya (Cyprinuscarpio carpio) Menggunakan Metode
349 RAPD. Indonesian. 2012; 3(3): 15–23.

350 [38] Yustiati A, Hutagalung RM, Andriani Y, Lili W, Bangkit I. Genetic Analysis of Tilapia
351 (Oreochromis niloticus) Nirwana Strain Cultured in Aceh Besar and Wanayasa by Using
352 Random Amplified Polymorphic DNA Method. Asian Journal of Biochemistry, Genetics and
353 Molecular Biology, February 2021, 2020.;4(4)1–9.

354 [39] Gusmiaty G, Restu M, Asrianny A, Larekeng SH. Polimorfisme Penanda RAPD untuk
355 Analisis Keragaman Genetik Pinusmerkusii di Hutan PendidikanUnhas. Jurnal Natur
356 Indonesia. Indonesian. 2017;16(2): 47-53.

357 [40] Nei M. Molecular Evolutionary Genetics. New York: Columbia University Press. 1987.

358 [41] White W, Last P, Stevens J, Yearsley G, Fahmi, Dharmadi. Economically important
359 sharks and rays of Indonesia. Australian Centre for International Agricultural Research;
360 2006.

361 [42] Jannah M, Sari NK, Mushlih M, Rifqi M, Priyambodo, Pratiwi, RH, et.al. Metode Biologi
362 Molekuler. Widina Bhakti Persada Bandung. Indonesian; 2021.

363 [43] Yudianto A. Cell Free Fethal DNA Metode Non Invasive dalam Pemeriksaan Identifikasi.
364 Scopindo Media Pustaka. Indonesian; 2019.

365 [44] White WT, Corrigan S, Yang L, Henderson AC, Bazinet AL, Swofford DL, Naylor GJP.
366 Phylogeny of the manta and devilrays (Chondrichthyes: Mobulidae), with an updated
367 taxonomic arrangement for the family. Zoological Journal of the Linnean Society,
368 2018;182(1): 50–75.

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